formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block de novo initiation of RNA synthesis.

391-Pos

Simulating the Relaxation of DNA Supercoils By Topoisomerase I

Todd D. Lillian[1], Maryna Taranova[2], Ioan Andricioaei[2], Noel C. Perkins[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California - Irvine, Irvine, CA, USA.

Many cellular processes involving DNA, including replication and transcription, result in significant superhelical stresses. During transcription, for example, RNA polymerase locally untwists about a helical turn of the DNA double helix. Then to elongate the RNA transcript, it proceeds along the template strand of the DNA and thereby induces supercoiling. DNA topoisomerases play an important role in relieving these stresses. Here we focus on understanding the action of human DNA topoisomerase I (Topo1) which operates in three basic steps: (i) cleaving a single strand of the DNA double helix, (ii) allowing the DNA superhelical stresses to relax, and (iii) religating the DNA. Recently, the Dekker lab, at Delft University of Technology, performed single molecule experiments to probe the relaxation of supercoils by topo1. A significant molecular dynamics (MD) effort (>100 cpu years) by the Andricioaei lab, at the University of California Irvine, characterized the energetics and topological changes of topo1 in complex with only a short fragment of DNA (~20 bp). Including a longer length of DNA to represent a biologically relevant length-scale (greater than a persistence length), is computationally prohibitive for MD and was necessarily neglected. Here we introduce an elasto-dynamic rod model as a first approximation to provide a dynamic description of the DNA as it relaxes. The rod model describes bending and torsion of the DNA helical axis, electrostatic and self-contact interactions, and approximates the hydrodynamic drag on the molecule. For our simulations, we provide as initial conditions, a plectomemic supercoil. The MD simulations serve to provide boundary conditions to the rod model by characterizing the torque applied to the DNA by topo1 as it rotates. Here we present preliminary results for the relaxation rates of supercoils.

392-Pos

Nucleoprotein Complex Formation By *Bacillus Subtilis* Spo0J/ParB

Candice M. Etson[1], David Z. Rudner[2], Antoine M. van Oijen[2].
[1]Harvard University/Harvard Medical School, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA.

Although prokaryotes lack the machinery utilized by eukaryotes to achieve well organized chromosome segregation, all cells must faithfully segregate their chromosomes in every cell division cycle. In many bacteria, this process is dependent upon a partitioning locus composed of an ATPase called ParA, a DNA binding protein called ParB, and centromere-like binding sites (*parS*) that are present adjacent to the origin of replication. ParB binds the *parS*, sites and the ParA ATPase acts on the ParB-*parS* complex to facilitate segregation of replicated origins toward opposite cell poles. In *Bacillus subtilis*, the ParA protein is called Soj and the ParB protein is referred to as Spo0J. There are eight *parS* sites surrounding the origin of replication of the *B. subtilis* chromosome. Spo0J can bind to these sites and spread along the DNA up to 15 kilobases, forming a nucleoprotein complex. The Spo0J-*parS* complexes are not only a substrate for Soj/ParB, but they also serve to recruit the highly conserved structural maintenance of chromosomes (SMC) complex to the origin. The SMC condensin complex appears to function in both organizing the origin region and facilitating chromosome segregation. To investigate how Spo0J spreads along the DNA and ultimately how this complex recruits SMC, we have employed single molecule fluorescence imaging to directly observe the formation of the nucleoprotein complex of purified *B. subtilis* Spo0J and lambda DNA. We characterize the physical properties of this nucleoprotein complex and the kinetics of its formation.

393-Pos

Theoretical Analysis of the Molecular Mechanism of Stabilization of Nova-RNA Complex System: Fragment Molecular Orbital Method Based Quantum Chemical Calculation For the Effect of the Complex Formation on the Electronic State of Biomacromolecular System

Ikuo Kurisaki[1], Kaori Fukuzawa[2], Tatsuya Nakano[3], Yuji Mochizuki[4], Hirofumi Watanabe[1,5], Shigenori Tanaka[1,5].
[1]Kobe university, 3-11, Tsurukabuto, Nada, Kobe, Japan, [2]Mizuho Information & Research Institute, Inc, 2-3 Kanda Nishiki-cho, Chiyoda-ku, Tokyo, Japan, [3]National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, Japan, [4]Rikkyo University, 3-34-1 Nishi-Ikebukuro, Toshima-ku, Tokyo, Japan, [5]Japan Science and Technology Agency, CREST, 5 Sanban-cho, Chiyoda-ku, Tokyo, Japan.

The RNA-binding proteins (RBPs) specifically recognize the shape and/or sequence of RNA molecules for binding the target. The relationship between three-dimensional structure and RNA-binding mechanism of RBPs can be analyzed by performing computer simulations to provide deeper insight into this issue. In the present study, we performed Fragment Molecular Orbital (FMO) based quantum chemical calculations for neuro-oncological ventral antigen third KH domain (NOVA)-RNA complex system to study the molecular mechanism from the viewpoint of electronic state of biomacromolecules. We investigated the effect of the complex formation on an electronic state of NOVA. We found and that the charge redistributes all over the structure and that the secondary structure of NOVA is remarkably associated with the change of electronic state in the complex formation. The results indicate that the whole protein structure participates in realization of the best energetic stabilization in the complex formation and we speculate that secondary structure could play an important role to obtain the optimum inter-molecular interaction energy by associating with charge redistribution. Further, we employ molecular dynamics simulation method to consider structures fluctuating around the equilibrium state. We perform FMO calculations for the obtained snapshots and examine the change of electronic state. The results will provide deeper insight into the relationship between electronic state and structural fluctuation. The details will be reported at the meeting.

394-Pos

Investigating Classic Lac Repressor-Dna Looping Experiments Using a Computational Rod Model

Andrew D. Hirsh, Todd D. Lillian, Noel C. Perkins.
University of Michigan - Ann Arbor, Ann Arbor, MI, USA.

Protein mediated DNA looping is a well known gene regulatory mechanism. A commonly studied system that controls gene expression is lactose repressor (LacI) induced DNA looping. In two in vitro studies, the Müller-Hill group investigates how the lac repressor protein in E. coli forms loops with linear and cyclized DNA. Their experiments analyzed LacI induced looping on linear DNA over a wide range of interoperator lengths (6-21 helical turns) and on supercoiled DNA minicircles of 452 base pairs. In these experiments, electron microscopy, non-denaturing polyacrylamide gel elecrophoresis, and DNase I protection experiments were used to detect loop formation, estimate loop size, quantify loop stability, and for supercoiled DNA to detect loop topology (ΔLk). In our study, we exercise our computational rod model to make side-by-side comparisons of our predictions with their experimental observations. By making comparisons, we look to understand the energetic cost of loop formation and the resulting topology of the looped complex.

395-Pos

High Throughput Screening of Aptamers For Human Thrombin and Factor IXa

Gillian V. Kupakuwana[1], Lei Chen[1], James E. Crill[2], Mark P. McPike[2], Philip N. Borer[1].
[1]Syracuse University, Syracuse, NY, USA, [2]OrthoSystems, Inc., Syracuse, NY, USA.

The traditional method for discovery of DNA/RNA aptamers is *in vitro* evolution (SELEX), where multiple cycles of partitioning and amplification enrich aptamer candidates from a pool containing randomized segments of length, m. Previous to our work the consensus sequence for alpha-thrombin aptamers (ThbA) was found via five rounds of SELEX in a DNA pool with m=60, while factor IXa aptamers (FIXaA) were discovered in an RNA library of m=40 after 8 rounds. We isolated the same ThbA, FIXaA and a novel carbohydrate aptamer (CA) to validate our new method, High Throughput Screening of Aptamers. HTSA uses a single partitioning step, PCR, and counts survivors by massively parallel sequencing. We found the minimal ThbA in a library of DNA hairpins loops (m=15) containing 56,000 copies of each of the 1.1 billion possible sequences. We distinguished two sequence motifs well above the background. The ThbA motif contains the consensus (counted 46,000 times) and dozens of related sequences. The leading candidate in the CA family (29,000 counts) is a novel aptamer that binds glucose (Kd=1,400 nM) and alpha-methyl mannoside (Kd=500 nM). A known FIXaA was counted 52,000 times from a library of RNA hairpins (m=16 with 14,000 copies of each sequence). HTSA simplifies and shortens the discovery process, exhaustively searches the space of sequences within a library, simplifies characterization of the core binding domain, reduces the quantity of the target required, eliminates cycling artifacts, allows multiplexing of targets, and requires no complex automation.

396-Pos

Engineered Holliday Junctions As Single-Molecule Reporters For Protein-Dna Interactions

Nesha May O. Andoy, Susanta Sarkar, Peng Chen.
Cornell University, Ithaca, NY, USA.

Protein-DNA interactions are essential for gene replication and expression. Characterizing how proteins interact with and change the structure of DNA